apparently due to concerns about certain aspects of the present invention as set out in the Advisory Action.

In particular, the examiner has cited prior art document '228 and has concluded that this document discloses the use of supercoiled DNA in a method for assaying the DNA excision and resynthesis method of a biological solution. As pointed out in the previous response, however, this is not correct because '228 does not use supercoiled DNA molecules, but instead uses either non-supercoiled short single or double stranded oligonucleotides which can be either synthesised for instance by PCR or generated by digestion of a starting material such as a plasmid. The materials used in '228, therefore, are not supercoiled and nor indeed are they plasmids.

Although '228 addresses an arguably similar problem to the claimed invention, it does so in a very different way; in '228 the authors provide short bound single of double stranded oligonucleotides of known sequence which contain one or more defects such as a base mismatch or apurinic site, mimicking a mutation which requires excision and resynthesis repair. Samples are then applied to the bound oligos and the incorporation of labelled nucleotides is used to determine the repair capacity of the tested sample. Such an approach has advantages with respect to the prior art, namely it only measures DNA repair mechanisms which act upon the selected mutations which diminishes the recorded effects of other repair mechanisms.

This is not the same approach as the present invention however, which uses altered supercoiled plasmids to test the repair capacities of a biological sample or medium. These altered plasmids are purified so that only supercoiled plasmids are isolated, which means that plasmids which have undergone a strand break or which more generally have a damaged structure and have probably been acted upon by a nuclease are eliminated. The remaining supercoiled plasmids which comprise a random assortment of other types of mutations and

lesions which require DNA excision and resynthesis and therefore can act as a reagent upon which the excision and resynthesis repair capacities of the biological medium can be measured.

The method according to the present invention is different from and actually better than the one described in '228 as although the exact mutations/lesions are not known in the claimed invention for the mutated supercoiled plasmids used, because the target reagent (the plasmids in claim 22) contains a large number of mutation types in a wide variety of locations, such a heterogeneous target reagent, can more accurately quantify all the various excision and resynthesis repair capacities of the biological medium, than can a target reagent which consists of a more homogeneous/single mutation type (as in '228).

Therefore the method recited in claim 22 has involved an inventive step (and is thus patentable) with respect to '228, as starting from '228 the skilled man would have thought to use supercoiled plasmid DNA as the target reagent but instead would have followed the teaching of '228 and used a set of short oligos, potentially generated from a starting plasmid DNA source by PCR or restriction endonuclease digestion, which are not supercoiled or indeed plasmids.

B. Plasmid DNA

The Examiner states that '228 contemplates the use of plasmid DNA, because of the statement at paragraph 88 of '228 referred to in the Advisory Action. In paragraph 88, it is specified that the DNA used in 'linear plasmid DNA'. To interpret the meaning of this term, it is necessary to look at the rest of the description of '228; the term plasmid is used only in paragraphs 88, 91 and 93 of '228. In paragraphs 88 and 93 examples of DNA matrixes with specific DNA matrixes are DNA fragments generated by PCR using primers which contain NH_2 at their 5' end, wherein the template is plasmid DNA (paragraph 92). The second source

of DNA molecules is to cleave plasmids using a restriction endonuclease to get two shorter linear plasmid fragments which in turn each contain NH_2 at their 5' end (paragraph 93).

The first DNA molecule(s) (paragraph 92) is not plasmid DNA but instead is the amplification product of a PCR on the plasmid template.

The second DNA molecule(s) (paragraph 93) is a linear fragment of plasmid DNA.

C. Supercoiled

The examiner states that You et al discloses that plasmid DNA is supercoiled. It is assumed the examiner is basing this statement of the first paragraph of You et al wherein it is stated "Because almost all DNA in living cells is (-) supercoiled...". It is not disputed that, in general, all DNA *in vivo* is present in a partially or fully supercoiled state, in fact this very point has been made in previous responses.

However, '228 does not describe plasmid DNA *in vivo*, rather in '228 linear fragments of plasmid DNA are generated *in vivo* either by PCT or restriction digestion, see above.

Hence, Applicant contends not that plasmid DNA is not generally supercoiled, this point was explicitly stipulated in the last response and is admitted again herein, but instead that '228 does not teach or suggest the use of supercoiled plasmid DNA *in vitro.*

Morever, it is assumed that the examiner accepts the previous arguments that to introduce into a DNA molecule a supercoil requires the expenditure of ATP and the action of one or more enzymes such as topoisomerases upon the DNA molecule. Firstly, therefore, even assuming a supercoil could be introduced into a linear double strand DNA PCR product, '228 does not disclose or suggest that this should be done. Secondly, when a strand of a supercoiled DNA molecule is broken (for instance by cleavage with an endonuclease) either the nicked (that is DNA which has one strand cut) or linearised (both DNA strands cut)

plasmids undergo structural changes which lead to the well known differences in migration through an agarose gel of supercoiled vs. nicked vs. linear versions of a plasmid.

These differences in migration specifically result from the change in the plasmid DNA molecule from a closed circular molecule of double stranded DNA which is coiled upon itself due to the introduced internal tensions (supercoiled), to an open circular double stranded DNA molecule, which do not have any introduced internal tensions. Specifically these changes from one structure to another, occur due to breaks in the phosphate backbone of one or more DNA strands which once made allow the unravelling of the DNA molecule relieving the internal tensions therein.

These principles are so well known in the art, that it is difficult to provide a single reference which sets these points out. Hence, a Declaration Under Rule 132 was submitted with the previous response, in which the inventor Dr. Sauvaigo, who is an expert in this field, addressed these points in paragraphs 6 to 14 of the Declaration.

To illustrate further this point concerning the differences between supercoiled and no-supercoiled plasmid DNA, please find enclosed an extract from the Qiagen® Plasmid Purification Handbook (Third Edition, November 2005, Qiagen) and specifically page 42 thereof which shows an agarose gel analysis of plasmid DNAs and which in lanes L, E and 1 to 4, shows that supercoiled plasmid DNA migrates faster than the open circular plasmid DNA (that is non-supercoiled plasmid DNA).

As attested by Dr. Sauvaigo in her Declaration and as known in the art (see previous paragraph), following either the intentional or unintentional breaking of one or both strands of a supercoiled DNA molecule, this DNA molecule will relax so losing its supercoiled structure.

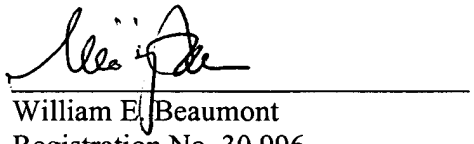
Coming back to the disclosure of '228, this prior art reference discloses using endonuclease derived linear fragments of a plasmid (which may or may not have been in a

supercoiled form prior to digestion). These linear fragments are not supercoiled because of the strand breaks introduced into the plasmid by the endonuclease (and/or in the case of PCR products because no manipulations were made to the products following their synthesis/cleavage).

Therefore, although '228 contemplates the use of linear DNA fragments derived from plasmid DNA either by PCR or endonuclease digestion, neither of these DNA fragment types are supercoiled and hence the present method which uses intact supercoiled plasmid DNA molecules is both novel and patentable, for this reason at least, with respect to all the prior art documents cited by the examiner.

Favorable consideration is earnestly solicited.

Respectfully submitted,
THE NATH LAW GROUP



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Third Edition November 2005

QIAGEN® Plasmid Purification Handbook

QIAGEN Plasmid Mini, Midi, Maxi, Mega, and Giga Kits

For purification of ultrapure, transfection grade plasmid DNA

WWW.QIAGEN.COM

Contents

Kit Contents

QIAGEN Plasmid Kit	Mini (25)	Mini (100)	Midi (25)	Midi (100)	Maxi (10)	Maxi (25)	Maxi (100)
Catalog no.	**12123**	**12125**	**12143**	**12145**	**12162**	**12163**	**12165**
QIAGEN-tip 20	25	100	–	–	–	–	–
QIAGEN-tip 100	–	–	25	100	–	–	–
QIAGEN-tip 500	–	–	–	–	10	25	4 x 25
Buffer P1	20 ml	40 ml	110 ml	440 ml	110 ml	280 ml	4 x 280 ml
Buffer P2	20 ml	40 ml	110 ml	440 ml	110 ml	280 ml	4 x 280 ml
Buffer P3	20 ml	40 ml	110 ml	440 ml	110 ml	280 ml	4 x 280 ml
Buffer QBT	40 ml	110 ml	110 ml	440 ml	110 ml	280 ml	4 x 280 ml
Buffer QC	120 ml	480 ml	3 x 190 ml	3 x 750 m	3 x 240 ml	4 x 425 ml	16 x 425 ml
Buffer QF	30 ml	110 ml	170 ml	2 x 280 ml	170 ml	420 ml	4 x 420 ml
RNase A*	2 mg	4 mg	11 mg	44 mg	11 mg	28 mg	4 x 28 mg
LyseBlue	20 µl	40 µl	110 µl	440 µl	110 µl	280 µl	4 x 280 µl
Handbook	1	1	1	1	1	1	1

* Provided in a 10 mg/ml or 100 mg/ml solution.

QIAGEN Plasmid Kit	Mega (5)	Mega (25)	Giga (5)	Plasmid Buffer Set
Catalog no.	**12181**	**12183**	**12191**	**19046**
QIAGEN-tip 2500	5	25	5	–
QIAGEN-tip 10000	–	–	–	–
Buffer P1	2 x 140 ml	2 x 700 ml	700 ml	110 ml
Buffer P2	2 x 140 ml	2 x 700 ml	700 ml	110 ml
Buffer P3	2 x 140 ml	2 x 700 ml	700 ml	110 ml
Buffer QBT	200 ml	2 x 500 ml	400 ml	110 ml
Buffer QC	5 x 220 ml	5 x 1000 ml, 1 x 500 ml	3 x 1000 ml, 1 x 500 ml	6 x 120 ml
Buffer QF	200 ml	2 x 510 ml	1 x 510 ml	2 x 85 ml
RNase A*	2 x 14 mg	2 x 70 mg	70 mg	11 mg
LyseBlue	2 x 140 µl	2 x 700 µl	700 µl	110 µl
Handbook	1	1	1	1

* Provided in a 10 mg/ml or 100 mg/ml solution.

Storage

QIAGEN-tips should be stored dry and at room temperature (15–25°C). They can be stored for at least 2 years without showing any reduction in performance, capacity, or quality of separation.

QIAGEN Plasmid Kits should be stored at room temperature (15–25°C). After adding RNase A, Buffer P1 should be stored at 2–8°C and is stable for 6 months. Other buffers and RNase A stock solution can be stored for 2 years at room temperature (15–25°C).

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of QIAGEN Plasmid Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

QIAGEN Plasmid Kits are developed, designed and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Service or your local distributor (see back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding any aspect of QIAGEN, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Comprehensive background information on plasmid preparation procedures and common plasmid applications — in addition to kit selection guides, frequently asked questions, and information about our purification technologies — can be found on our plasmid Web page www.qiagen.com/goto/plasmidinfo .

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

The following risk and safety phrases apply to components of the QIAGEN Plasmid Kits.

Buffer P2

Contains sodium hydroxide: irritant. Risk and safety phrases:* R36/38, S13-26-36-46

Buffer P3

Contains acetic acid: irritant. Risk and safety phrases:* R36/38, S13-26-36-46

Buffers QBT, QC, QF

Contain isopropanol: flammable. Risk and safety phrases:* R10

RNase A

Contains ribonuclease: sensitizer. Risk and safety phrases:* R42/43, S23-24-26-36/37

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R10: Flammable; R36/38: Irritating to eyes and skin; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink, and animal feedingstuffs; S23: Do not breathe vapor; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37: Wear suitable protective clothing and gloves; S46: If swallowed, seek medical advice immediately and show this container or label.

Introduction

QIAGEN Plasmid Purification Kits are based on the remarkable selectivity of patented QIAGEN Resin, allowing purification of ultrapure supercoiled plasmid DNA with high yields.

Anion-exchange–based QIAGEN-tips yield transfection grade DNA, which is highly suited for use in a broad variety of demanding applications such as transfection, in vitro transcription and translation, and all enzymatic modifications. QIAGEN offers the most comprehensive portfolio of tailored plasmid purification kits for any scale, throughput, or downstream application. Select the optimum kit for your requirements by visiting our online selection guide at www.qiagen.com/products/plasmid/selectionguide . For transfection, QIAGEN also offers the advanced PolyFect®, SuperFect®, and Effectene® transfection reagents. These reagents, combined with the high-quality plasmid DNA obtained from QIAGEN, QIAfilter, HiSpeed®, and EndoFree® Plasmid Kits, provide optimal transfection results (for ordering information, see pages 46 – 49).

Principle and procedure

QIAGEN plasmid purification protocols are based on a modified alkaline lysis procedure, followed by binding of plasmid DNA to QIAGEN Anion-Exchange Resin under appropriate low-salt and pH conditions. RNA, proteins, dyes, and low-molecular-weight impurities are removed by a medium-salt wash. Plasmid DNA is eluted in a high-salt buffer and then concentrated and desalted by isopropanol precipitation.

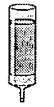
Each disposable QIAGEN-tip packed with QIAGEN Resin is designed to operate by gravity flow, reducing the amount of hands-on time required for the purification procedure.

QIAGEN Plasmid Kits

Pelleted bacteria

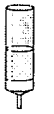
Alkaline lysate

Clear lysate
by centrifugation



Bind DNA

Wash



Elute

Isopropanol precipitate

Ultrapure plasmid DNA

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

For all protocols:

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, 37°C shaking incubator, and centrifuge with rotor and tubes or bottles for harvesting cells)
- QIArack or equivalent holder (see "Setup of QIAGEN-tips", page 13)
- Ice
- Isopropanol
- 70% ethanol
- Plasmid resuspension buffer (e.g., TE buffer, pH 8.0, or Tris·Cl, pH 8.5)

For QIAGEN Plasmid Mini Kit protocol:

- Microcentrifuge
- 1.5 ml or 2 ml microcentrifuge tubes

For QIAGEN Plasmid Midi, Maxi, Mega, and Giga Kit protocols:

- Centrifugation tubes or vessels with suitable capacity for the volumes specified in the appropriate protocol.
- Refrigerated centrifuge capable of ≥20,000 x *g* with rotor for the appropriate centrifuge tubes or bottles

Important Notes

Please take a few moments to read this handbook carefully before beginning the DNA preparation. If QIAGEN Plasmid Purification Kits are new to you, please visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results". Also be sure to read and follow the appropriate detailed protocol.

Plasmid size

Plasmids up to approximately 150 kb can be purified using QIAGEN plasmid purification protocols. Constructs larger than 45–50 kb, however, may exhibit somewhat reduced elution efficiencies. Prewarming the elution buffer to 65°C may help to increase the yield of large plasmids. For the isolation of large cosmid and plasmid DNA constructs, the QIAGEN Large-Construct Kit is available (see ordering information on page 48).

Plasmid/cosmid copy number

Plasmid and cosmids vary in copy number, depending on the origin of replication they contain, their size, and the size of insert. The protocols in this handbook are grouped according to the copy number of the plasmid or cosmid to be purified. High- and low-copy plasmids and cosmids should be purified using the standard protocols on pages 15 – 28. Very low-copy plasmids and very low-copy cosmids (<10 copies per cell) should be purified using the protocol on page 29, which uses extremely large culture volumes to obtain good yields. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

Host strains

The strain used to propagate a plasmid can have a substantial influence on quality of the purified DNA. Host strains such as DH1, DH5α®, and C600 yield high-quality DNA with QIAGEN protocols. The slower growing strain XL1-Blue also yields DNA of very high quality.

Strain HB101 and its derivatives, such as TG1 and the JM100 series, contain large amounts of carbohydrates that are released during lysis and can inhibit enzyme activities if not completely removed. In addition, some strains, such as JM101, JM110, and HB101, have high levels of endonuclease activity and yield DNA of lower quality. If the quality of purified DNA is not as expected, a change of host strain should be considered. If difficulty is encountered with strains such as TG1 and Top10F, we recommend either reducing the amount of culture volume or doubling the volumes of Buffers P1, P2, and P3 in order to improve the ratio of biomass to lysis buffers for optimized lysis conditions.

Table 1. Origins of Replication and Copy Numbers of Various Plasmids and Cosmids

DNA construct	Origin of replication	Copy number	Classification
Plasmids			
pUC vectors	pMB1*	500–700	high copy
pBluescript® vectors	ColE1	300–500	high copy
pGEM® vectors	pMB1*	300–400	high copy
pTZ vectors	pMB1*	>1000	high copy
pBR322 and derivatives	pMB1*	15–20	low copy
pACYC and derivatives	p15A	10–12	low copy
pSC101 and derivatives	pSC101	~5	very low copy
Cosmids			
SuperCos	pMB1	10–20	low copy
pWE15	ColE1	10–20	low copy

* The pMB1 origin of replication is closely related to that of ColE1 and falls in the same incompatibility group. The high-copy plasmids listed here contain mutated versions of this origin.

Culture media

QIAGEN plasmid purification protocols are optimized for use with cultures grown in standard Luria Bertani (LB) medium to a cell density of approximately 3–4 x 10^9 cells/ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium. Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are commonly used. We recommend growing cultures in LB medium containing 10 g NaCl per liter (Table 2) to obtain the highest plasmid yields.

Rich media are not recommended for plasmid preparation with QIAGEN-tips. If rich media must be used, growth time must be optimized, and culture volumes reduced. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

Table 2. Composition of Luria Bertani Medium

Contents	per liter
Tryptone	10 g
Yeast extract	5 g
NaCl	10 g

Please refer to Appendix B on page 44 for preparation of LB medium.

Culture volume

Do not exceed the maximum recommended culture volumes given at the beginning of each protocol (and on the card inside the back cover of this handbook). Using larger culture volumes will lead to an increase in biomass and can affect the efficiency of alkaline lysis, leading to reduced yield and purity of the preparation.

The protocol for QIAGEN Plasmid Kits is optimized for use with cultures grown in standard Luria Bertani (LB) medium (see page 12), grown to a cell density of approximately 3–4 x 10^9 cells per ml. We advise harvesting cultures after approximately 12–16 hours of growth, which typically is the transition from logarithmic into stationary growth phase. It is best to assess the cell density of the culture and, if that is too high, to reduce the culture volumes accordingly or increase the volumes of Buffers P1, P2, and P3. A high ratio of biomass to lysis buffers will result in poor lysis conditions and subsequently low DNA yield and purity. For determination of cell density, calibration of each individual spectrophotometer is required to facilitate accurate conversion of OD_{600} measurements into the number of cells per milliliter. This can be achieved by plating serial dilutions of a culture onto LB-agar plates in the absence of antibiotics. The counted colonies are used to calculate the number of cells per milliliter, which is then set in relation to the measured OD_{600} values.

Capacity of QIAGEN-tips

QIAGEN-tips are available in a variety of sizes for preparation of as little as 20 µg or as much as 10 mg plasmid DNA (Figure 1, next page). The maximum plasmid binding capacities of the QIAGEN-tips 20, 100, 500, 2500, and 10000 are at least 20 µg, 100 µg, 500 µg, 2.5 mg, and 10 mg, respectively. Actual yields will depend on culture volume, culture medium, plasmid copy number, size of insert, and host strain. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

Setup of QIAGEN-tips

QIAGEN-tips may be held upright in a suitable collection vessel such as a tube or flask, using the tip holders provided with the kits (Figure 2A). Alternatively, the QIAGEN-tips 20, 100, 500, and 2500 may be placed in the QIArack (cat. no. 19015) (Figure 2B).

Analytical gel analysis

The success of the plasmid purification procedure can be monitored on an analytical gel (see Figure 3, page 42). We recommend removing and saving aliquots where indicated during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification the problem occurred (see page 41).

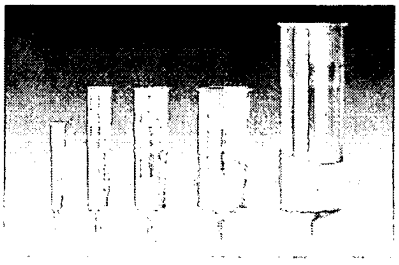


Figure 1 QIAGEN-tip 20 to QIAGEN-tip 10000.

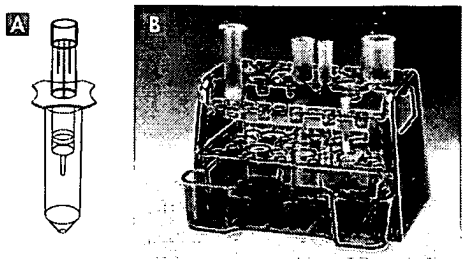


Figure 2 Setup of QIAGEN-tips **A** with tip holder or **B** with the QIArack.

Convenient stopping points in protocols

For all protocols, the purification procedure can be stopped and continued later by freezing the cell pellets obtained by centrifugation. The frozen cell pellets can be stored at –20°C for several weeks. In addition, the DNA eluted from the QIAGEN-tip can be stored overnight at 2–8°C,* after which the protocol can be continued. These stopping points are indicated by the symbol ⊗.

Using LyseBlue reagent

LyseBlue is a color indicator which provides visual identification of optimum buffer mixing. This prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. This makes LyseBlue ideal for use by researchers who have not had much experience with plasmid preparations as well as experienced scientists who want to be assured of maximum product yield.

LyseBlue can be added to the resuspension buffer (Buffer P1) bottle before use. Alternatively, smaller amounts of LyseBlue can be added to aliquots of Buffer P1, enabling single plasmid preparations incorporating visual lysis control to be performed.

LyseBlue reagent should be added to Buffer P1 at a ratio of 1:1000 to achieve the required working concentration (e.g., 10 µl LyseBlue into 10 ml Buffer P1). Make sufficient LyseBlue/Buffer P1 working solution for the number of plasmid preps being performed.

LyseBlue precipitates after addition into Buffer P1. This precipitate will completely dissolve after addition of Buffer P2. Shake Buffer P1 before use to resuspend LyseBlue particles.

The plasmid preparation procedure is performed as usual. After addition of Buffer P2 to Buffer P1, the color of the suspension changes to blue. Mixing should result in a homogeneously colored suspension. If the suspension contains localized regions of colorless solution or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

Upon addition of neutralization buffer (Buffer P3 or Buffer N3), LyseBlue turns colorless. The presence of a homogeneous solution with no traces of blue indicates that SDS from the lysis buffer has been effectively precipitated.

* Longer storage is not recommended.

Protocol: Plasmid or Cosmid DNA Purification Using QIAGEN Plasmid Mini Kit

This protocol is designed for preparation of up to 20 µg of high-copy plasmid or cosmid DNA using the QIAGEN Plasmid Mini Kit. For additional protocols, such as for cosmid, low-copy-number plasmid, BACs, PACs, P1s, and double-stranded M13 replicative form purification, see the recommendations at www.qiagen.com/goto/plasmidinfo .

Important notes before starting

- New users are advised to familiarize themselves with the detailed protocol provided in this handbook. In addition, extensive background information is provided on our plasmid resource page www.qiagen.com/goto/plasmidinfo .
- Optional: Remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel (see page 41)

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (centrifuge briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Pre-chill Buffer P3 at 4°C.
- Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use one vial LyseBlue (centrifuge briefly before use) per bottle of Buffer P1 to achieve a 1:1000 dilution. LyseBlue provides visual identification of optimum buffer mixing thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details see "Using LyseBlue reagent" on page 14.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approximately 8 h at 37°C with vigorous shaking (approx. 300 rpm).**

   Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. **Dilute the starter culture 1/500 to 1/1000 into 3 ml selective LB medium. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).**

   Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per milliliter, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium.

3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**

   ⊗ If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.

4. **Resuspend the bacterial pellet in 0.3 ml of Buffer P1.**

   Ensure that RNase A has been added to Buffer P1.

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

5. **Add 0.3 ml of Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) for 5 min.**

   Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from CO_2 in the air.

   If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

6. **Add 0.3 ml of chilled Buffer P3, mix immediately and thoroughly by vigorously inverting 4–6 times, and incubate on ice for 5 min.**

   Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and KDS. The lysate should be mixed thoroughly to ensure even potassium dodecyl sulphate precipitation. If the mixture still appears viscous, more mixing is required to completely neutralize the solution.

   If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

7. **Centrifuge at maximum speed in a microcentrifuge for 10 min. Remove supernatant containing plasmid DNA promptly.**

   Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed at maximum speed in 1.5 ml or 2 ml microcentrifuge tubes (e.g., 10,000–13,000 rpm in a microcentrifuge). Maximum speed corresponds to 14,000–18,000 x *g* for most microcentrifuges. After centrifugation, the supernatant should be clear. If the supernatant is not clear, a second, shorter centrifugation should be carried out to avoid applying any suspended or particulate material to the column. Suspended material (which causes the sample to appear turbid) will clog the column and reduce or eliminate flow.

   ☞ Remove a 50 µl sample from the cleared lysate and save it for an analytical gel (sample 1).

8. **Equilibrate a QIAGEN-tip 20 by applying 1 ml Buffer QBT, and allow the column to empty by gravity flow.**

   Place QIAGEN-tips into a QIArack over the waste tray or use the tip holders provided with each kit (see "Setup of QIAGEN-tips" page 13). Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

9. **Apply the supernatant from step 7 to the QIAGEN-tip 20 and allow it to enter the resin by gravity flow.**

   The supernatant should be loaded onto the QIAGEN-tip promptly. If it is left too long and becomes cloudy due to further precipitation of protein, it must be centrifuged again before loading to prevent clogging of the QIAGEN-tip.

   ☞ Remove a 50 µl sample of the flow-through and save for an analytical gel (sample 2).

10. **Wash the QIAGEN-tip 20 with 2 x 2 ml Buffer QC.**

    Allow Buffer QC to move through the QIAGEN-tip by gravity flow.

    ☞ Remove a 220 µl sample of the combined wash fractions and save for an analytical gel (sample 3).

11. **Elute DNA with 0.8 ml Buffer QF.**

    Collect the eluate in a 1.5 ml or 2 ml microcentrifuge tubes (not supplied).

    Note: For constructs larger than 45–50 kb, prewarming the elution buffer to 65°C may help to increase yield.

    ☞ Remove a 45 µl sample of the eluate and save for an analytical gel (sample 4).

12. **Precipitate DNA by adding 0.7 volumes (0.56 ml per 0.8 ml of elution volume) of room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥10,000 rpm for 30 min in a microcentrifuge. Carefully decant the supernatant.**

    All solutions should be at room temperature in order to minimize salt precipitation. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

13. **Wash DNA pellet with 1 ml of 70% ethanol and centrifuge at 10,000 rpm for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

14. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10mM Tris·Cl, pH 8.5)**

    Redissolve the DNA pellet by rinsing the walls to recover all the DNA. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under slightly alkaline conditions; it does not easily dissolve in acidic buffers.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel. For reliable spectrophotometric DNA quantification, A_{260} readings should lie between 0.1 and 1.0.

Agarose gel analysis

We recommend removing and saving aliquots during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (see page 41).

Protocol: Plasmid or Cosmid DNA Purification Using QIAGEN Plasmid Midi and Maxi Kits

This protocol is designed for preparation of up to 100 µg of high- or low-copy plasmid or cosmid DNA using the QIAGEN Plasmid Midi Kit, or up to 500 µg using the QIAGEN Plasmid Maxi Kit. For additional protocols, such as for purification of very low-copy plasmids or cosmids of less than 10 copies per cell, see page 29 or visit www.qiagen.com/goto/plasmidinfo .

Low-copy plasmids that have been amplified in the presence of chloramphenicol should be treated as high-copy plasmids when choosing the appropriate culture volume.

Maximum Recommended Culture Volumes*

	QIAGEN-tip 100	QIAGEN-tip 500
High-copy plasmids	25 ml	100 ml
Low-copy plasmids	100 ml	500 ml

* For the QIAGEN-tip 100, the expected yields are 75–100 µg for high-copy plasmids and 20–100 µg for low-copy plasmids. For the QIAGEN-tip 500, the expected yields are 300–500 µg for high-copy plasmids and 100–500 µg for low-copy plasmids.

Important points before starting

- New users are advised to familiarize themselves with the detailed protocol provided in this handbook. In addition, extensive background information is provided on our plasmid resource page www.qiagen.com/goto/plasmidinfo .
- If working with low-copy vectors, it may be beneficial to increase the lysis buffer volumes in order to increase the efficiency of alkaline lysis, and thereby the DNA yield. In case additional Buffers P1, P2, and P3 are needed, their compositions are provided in Appendix B: Composition of Buffers, on page 44. Alternatively, the buffers may be purchased separately (see page 49).
- Optional: Remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel (see page 41).
- Blue (marked with a ▲) denotes values for QIAGEN-tip 100 using the QIAGEN Plasmid Midi Kit; red (marked with a ●) denotes values for QIAGEN-tip 500 using the QIAGEN Plasmid Maxi Kit.

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (centrifuge briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Pre-chill Buffer P3 at 4°C.
- Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use one vial LyseBlue (centrifuge briefly before use) per bottle of Buffer P1 to achieve a 1:1000 dilution. LyseBlue provides visual identification of optimum buffer mixing thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details see "Using LyseBlue reagent" on page 14.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).**

   Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. **Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids, inoculate ▲ 25 ml or ● 100 ml medium with ▲ 25–50 µl or ● 100–200 µl of starter culture. For low-copy plasmids, inoculate ▲ 100 ml or ● 500 ml medium with ▲ 100–200 µl or ● 250–500 µl of starter culture. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).**

   Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per milliliter, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium.

3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**

   ⊗ If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.

4. **Resuspend the bacterial pellet in ▲ 4 ml or ● 10 ml Buffer P1.**

   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1.

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

5. **Add ▲ 4 ml or ● 10 ml Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) for 5 min.**

   Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from CO_2 in the air.

   If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

6. **Add ▲ 4 ml or ● 10 ml of chilled Buffer P3, mix immediately and thoroughly by vigorously inverting 4–6 times, and incubate on ice for ▲ 15 min or ● 20 min.**

   Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and KDS. The lysate should be mixed thoroughly to ensure even potassium dodecyl sulfate precipitation. If the mixture still appears viscous, more mixing is required to completely neutralize the solution.

   If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed in non-glass tubes (e.g., polypropylene). After centrifugation the supernatant should be clear.

   Note: Instead of centrifugation steps 7 and 8, the lysate can be efficiently cleared by filtration using a QIAfilter Kits or Cartridges (see www.qiagen.com/products/plasmid/LargeScaleKits).

8. **Centrifuge the supernatant again at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   This second centrifugation step should be carried out to avoid applying suspended or particulate material to the QIAGEN-tip. Suspended material (causing the sample to appear turbid) can clog the QIAGEN-tip and reduce or eliminate gravity flow.

   ☞ Remove a ▲ 240 µl or ● 120 µl sample from the cleared lysate supernatant and save for an analytical gel (sample 1) in order to determine whether growth and lysis conditions were optimal.

9. **Equilibrate a ▲ QIAGEN-tip 100 or ● QIAGEN-tip 500 by applying ▲ 4 ml or ● 10 ml Buffer QBT, and allow the column to empty by gravity flow.**

   Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

10. **Apply the supernatant from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**

    The supernatant should be loaded onto the QIAGEN-tip promptly. If it is left too long and becomes cloudy due to further precipitation of protein, it must be centrifuged again or filtered before loading to prevent clogging of the QIAGEN-tip.

    ☞ Remove a ▲ 240 µl or ● 120 µl sample from the flow-through and save for an analytical gel (sample 2) in order to determine the efficiency of DNA binding to the QIAGEN Resin.

11. **Wash the QIAGEN-tip with ▲ 2 x 10 ml or ● 2 x 30 ml Buffer QC.**

    Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove all contaminants in the majority of plasmid DNA preparations. The second wash is especially necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

    ☞ Remove a ▲ 400 µl or ● 240 µl sample from the combined wash fractions and save for an analytical gel (sample 3).

12. **Elute DNA with ▲ 5 ml or ● 15 ml Buffer QF.**

    Collect the eluate in a 15 ml or 50 ml tube (not supplied). Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

    Note: For constructs larger than 45–50 kb, prewarming the elution buffer to 65°C may help to increase yield.

    ☞ Remove a ▲ 100 µl or ● 60 µl sample of the eluate and save for an analytical gel (sample 4).

    ⊗ If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

13. **Precipitate DNA by adding ▲ 3.5 ml or ● 10.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.**

    All solutions should be at room temperature in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. Alternatively, disposable conical bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

14. **Wash DNA pellet with ▲ 2 ml or ● 5 ml of room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    Alternatively, disposable conical-bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

15. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).**

    Redissolve the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under slightly alkaline conditions; it does not easily dissolve in acidic buffers.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel. For reliable spectrophotometric DNA quantification, A_{260} readings should lie between 0.1 and 1.0.

Agarose gel analysis

We recommend removing and saving aliquots during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (see page 41).

Protocol: Plasmid or Cosmid DNA Purification Using QIAGEN Plasmid Mega and Giga Kits

This protocol is designed for preparation of up to 2.5 mg of high- or low-copy plasmid or cosmid DNA using the QIAGEN Plasmid Mega Kit, or up to 10 mg using the QIAGEN Plasmid Giga Kit. For additional protocols, such as for purification of very low-copy plasmids or cosmids of less than 10 copies per cell, see page 29 or visit www.qiagen.com/goto/plasmidinfo .

Low-copy plasmids that have been amplified in the presence of chloramphenicol should be treated as high-copy plasmids when choosing the appropriate culture volume.

Maximum Recommended Culture Volumes*

	QIAGEN-tip 2500	QIAGEN-tip 10000
High-copy plasmids	500 ml (1.5 g pellet wet weight)†	2.5 liters (7.5 g pellet wet weight)†
Low-copy plasmids	2.5 liters (7.5 g pellet wet weight)†	5 liters‡‡ (15 g pellet wet weight)‡‡

* For the QIAGEN-tip 2500, the expected yields are 1.5–2.5 mg for high-copy plasmids and 0.5–2.5 mg for low-copy plasmids. For the QIAGEN-tip 10000, the expected yields are 7.5–10 mg for high-copy plasmids and 1–5 mg for low-copy plasmids.

† On average, a healthy 1 liter shaker culture yields a pellet with a wet weight of approximately 3 g. When working with fermentation cultures, however, the pellet wet weight may be significantly higher. Therefore, when using fermented cultures, please refer to the pellet wet weight instead of the recommended culture volumes.

‡ Requires doubled amounts of alkaline lysis buffers.

Important points before starting

- New users are advised to familiarize themselves with the detailed protocol provided in this handbook. In addition, extensive background information is provided on our plasmid resource page www.qiagen.com/goto/plasmidinfo .
- If working with low-copy vectors, it may be beneficial to increase the lysis buffer volumes in order to increase the efficiency of alkaline lysis, and thereby the DNA yield. In case additional Buffers P1, P2, and P3 are needed, their compositions are provided in Appendix B: Composition of Buffers, on page 44. Alternatively, the buffers may be purchased separately (see page 49).
- Optional: Remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel (see page 41).
- Blue (marked with a ▲) denotes values for QIAGEN-tip 2500 using the QIAGEN Plasmid Mega Kit; red (marked with a ●) denotes values for QIAGEN-tip 10000 using the QIAGEN Plasmid Giga Kit.

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (centrifuge briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Pre-chill Buffer P3 at 4°C.
- Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use one vial LyseBlue (centrifuge briefly before use) per bottle of Buffer P1 to achieve a 1:1000 dilution. LyseBlue provides visual identification of optimum buffer mixing thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details see "Using LyseBlue reagent" on page 14.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 5–10 ml LB medium containing the appropriate selective antibiotic. Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).**

   Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. **Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids, inoculate ▲ 500 ml or ● 2.5 liters medium with ▲ 500–1000 µl or ● 2.5–5 ml of starter culture. For low-copy plasmids, inoculate ▲ 2.5 liters or ● 5 liters medium with ▲ 2.5–5 ml or ● 5–10 ml of starter culture. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).**

   Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per milliliter, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium.

3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**

   Note: For Giga preparations of low-copy plasmids using 5 liters of culture, volumes of Buffers P1, P2, and P3 in steps 4–6 should be doubled, due to the very large number of cells harvested. For routine Giga preparation of low-copy plasmids, additional Buffers P1, P2, and P3 may need to be purchased (see page 49) or prepared (see page 44).

   ⊗ If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.

4. **Resuspend the bacterial pellet in ▲ 50 ml or ● 125 ml of Buffer P1.**

   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. We recommend a 500 ml bottle for Mega preparations and a 1000 ml bottle for Giga preparations. Ensure that the RNase A has been added to Buffer P1.

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

5. **Add ▲ 50 ml or ● 125 ml of Buffer P2, mix thoroughly by vigorously inverting 4–6 times, and incubate at room temperature for 5 min.**

   Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification of Buffer P2 from CO_2 in the air.

   If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

6. **Add ▲ 50 ml or ● 125 ml of chilled Buffer P3, mix immediately and thoroughly by vigorously inverting 4–6 times, and incubate on ice for 30 min.**

   Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and KDS. The lysate should be mixed thoroughly to avoid localized potassium dodecyl sulfate precipitation.

   If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed in 250 ml or 500 ml non-glass tubes (e.g., polypropylene; not supplied).

   Note: Instead of centrifugation steps 7 and 8, the lysate can be efficiently cleared by filtration using a QIAfilter Kits or Cartridges (see www.qiagen.com/products/plasmid/LargeScaleKits).

8. **Centrifuge the supernatant again at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   This step should be carried out to avoid applying suspended or particulate material to the QIAGEN-tip. Suspended material (causing the sample to appear turbid) can clog the QIAGEN-tip and reduce or eliminate gravity flow.

   ☞ Remove a ▲ 120 µl or ● 75 µl sample from the cleared lysate supernatant and save for an analytical gel (sample 1) in order to determine whether growth and lysis conditions were optimal.

9. **Equilibrate a ▲ QIAGEN-tip 2500 or ◍ QIAGEN-tip 10000 by applying ▲ 35 ml or ◍ 75 ml Buffer QBT, and allow the column to empty by gravity flow.**

   Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

10. **Apply the supernatant from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**

    The supernatant should be loaded onto the QIAGEN-tip promptly. If it is left too long and becomes cloudy due to further precipitation of protein, it must be centrifuged again or filtered before loading to prevent clogging of the QIAGEN-tip.

    ☞ Remove a ▲ 120 µl or ◍ 75 µl sample from the flow-through and save for an analytical gel (sample 2) in order to determine efficiency of DNA binding to the QIAGEN Resin.

11. **Wash the QIAGEN-tip with a total of ▲ 200 ml or a total of ◍ 600 ml Buffer QC.**

    Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first half of the volume of wash buffer is sufficient to remove all contaminants in the majority of plasmid DNA preparations. The second half is particularly necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

    ☞ Remove a ▲ 160 µl or ◍ 120 µl sample from the combined wash fractions and save for an analytical gel (sample 3).

12. **Elute DNA with ▲ 35 ml or ● 100 ml Buffer QF.**

    Use of polycarbonate centrifuge tubes for collection is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

    ☞ Remove a ▲ 22 µl or ● 20 µl sample of the eluate and save for an analytical gel (sample 4).

    Note: For constructs larger than 45–50 kb, prewarming the elution buffer to 65°C may help to increase yield.

    ⊗ If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

13. **Precipitate DNA by adding ▲ 24.5 ml or ● 70 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.**

    All solutions should be at room temperature in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. Alternatively, disposable conical bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

14. **Wash DNA pellet with ▲ 7 ml or ◎ 10 ml of room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    Alternatively, disposable conical-bottom centrifuge tubes (not supplied) can be used for centrifugation at 5000 x *g* for 60 min at 4°C. The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

15. **Air-dry the pellet for 10–20 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).**

    Redissolve the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under slightly alkaline conditions; it does not easily dissolve in acidic buffers.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel. For reliable spectrophotometric DNA quantification, A_{260} readings should lie between 0.1 and 1.0.

Agarose gel analysis

We recommend removing and saving aliquots during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (see page 41).

Protocol: Very Low-Copy Plasmid/Cosmid Purification Using QIAGEN-tip 100 or QIAGEN-tip 500

Very low-copy plasmids and cosmids of less than 10 copies per cell often require large culture volumes to yield significant amounts of DNA (for additional information see www.qiagen.com/goto/plasmidinfo). This protocol is suitable for QIAGEN-tip 100 or QIAGEN-tip 500. After alkaline lysis, there is an additional isopropanol precipitation step to decrease the amount of lysate before DNA is bound to the QIAGEN-tip. Culture volumes and tip sizes are selected to match the quantity of DNA expected to the capacity of the QIAGEN-tip. For purification of P1 and BAC DNA using QIAGEN-tips, please contact one of our technical service groups or your local distributor (see back cover). For purification of large cosmid and plasmid DNA constructs, for example, BAC, PAC, or P1 DNA, the QIAGEN Large-Construct Kit is available (see ordering information on page 48).

Details of yields, culture volumes, QIAGEN-tip sizes, and buffer volumes to be used for purification of very low-copy plasmids and cosmids are given in Table 3.

Table 3. Parameters for Purification of Very Low-Copy Plasmids and Cosmids of Less than 10 Copies Per Cell

Required DNA yield*	Up to 100 µg	Up to 500 µg
Culture volume	500 ml	2.5 liters
Buffer P1†	20 ml	125 ml
Buffer P2†	20 ml	125 ml
Buffer P3†	20 ml	125 ml
QIAGEN-tip	**QIAGEN-tip 100**	**QIAGEN-tip 500**
Buffer QBT (for equilibration)	4 ml	10 ml
Buffer QC (for washing)	2 x 10 ml	2 x 30 ml
Buffer QF (for elution)	5 ml	15 ml

* For very-low-copy plasmids, expected yields are 20–100 µg for the QIAGEN-tip 100 and 100–500 µg for the QIAGEN-tip 500.

† Volumes of lysis Buffers P1, P2, and P3 are higher than in the standard protocols on pages 19–23 in order to efficiently lyse the large number of cells required for purification of very low-copy plasmids and cosmids.

Important points before starting

- New users are advised to familiarize themselves with the detailed protocol provided in this handbook. In addition, extensive background information is provided on our plasmid resource page www.qiagen.com/goto/plasmidinfo .
- If working with low-copy vectors, it may be beneficial to increase the lysis buffer volumes in order to increase the efficiency of alkaline lysis, and thereby the DNA yield. In case additional Buffers P1, P2, and P3 are needed, their compositions are provided in Appendix B: Composition of Buffers, on page 41. Alternatively, the buffers may be purchased separately (see page 49).
- Optional: Remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel (see page 41).
- Blue (marked with a ▲) denotes values for QIAGEN-tip 100 using the QIAGEN Plasmid Midi Kit; red (marked with a ●) denotes values for QIAGEN-tip 500 using the QIAGEN Plasmid Maxi Kit.

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (centrifuge briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Pre-chill Buffer P3 at 4°C.
- Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use one vial LyseBlue (centrifuge briefly before use) per bottle of Buffer P1 to achieve a 1:1000 dilution. LyseBlue provides visual identification of optimum buffer mixing thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details see "Using LyseBlue reagent" on page 14.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–10 ml LB medium containing the appropriate selective antibiotic. Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).**

   Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. **Dilute the starter culture 1/500 to 1/1000 into ▲ 500 ml or ● 2.5 liters of selective LB medium using ▲ 500–1000 µl or ● 2.5–5 ml of starter culture. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).**

   Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium.

3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**

   ⊗ If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.

4. **Resuspend the bacterial pellet in ▲ 20 ml or ● 125 ml Buffer P1.**

   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1.

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspeded. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

5. **Add ▲ 20 ml or ● 125 ml Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) for 5 min.**

   Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification of Buffer P2 from CO_2 in the air.

   If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

6. **Add ▲ 20 ml or ● 125 ml chilled Buffer P3, mix immediately and thoroughly by vigorously inverting 4–6 times, and incubate on ice for 30 min.**

   Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and KDS. The lysate should be mixed thoroughly to avoid localized potassium dodecyl sulfate precipitation.

   If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed in non-glass tubes (e.g., polypropylene; not supplied). After centrifugation, the supernatant should be clear.

8. **Centrifuge the supernatant again at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly. Alternatively, the sample can be filtered over a prewetted, folded filter.**

   This second centrifugation step clears the lysate completely of precipitated material.

   ☞ Remove a ▲ 600 µl or ● 750 µl sample from the cleared lysate supernatant and save for an analytical gel (sample 1) to determine whether growth and lysis conditions were optimal.

9. **Precipitate the DNA by adding ▲ 42 ml or ● 262.5 ml (0.7 volumes) of room-temperature isopropanol to the lysate. Centrifuge at ≥15,000 x *g* for 30 min at 4°C, and carefully decant the supernatant.**

   This isopropanol precipitation reduces the sample volume to facilitate loading of the column. It also serves to remove unwanted metabolites such as proteins and lipopolysaccharides.

10. **Redissolve the DNA pellet in 500 µl TE buffer, pH 8.0, and add Buffer QBT to obtain a final volume of ▲ 5 ml or ● 12 ml for selected ▲ QIAGEN-tip 100 or ● QIAGEN-tip 500, respectively.**

    TE buffer is used to facilitate redissolving of the DNA. Buffer QBT provides optimal DNA binding conditions.

11. **Equilibrate a ▲ QIAGEN-tip 100 or ● QIAGEN-tip 500 by applying ▲ 4 ml or ● 10 ml Buffer QBT, and allow the column to empty by gravity flow.**

    Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

12. **Apply the DNA solution from step 10 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**

    ☞ Remove a ▲ 50 µl or ● 24 µl sample from the flow-through and save for an analytical gel (sample 2) in order to determine the efficiency of DNA binding to the QIAGEN Resin.

13. **Wash the QIAGEN-tip with ▲ 2 x 10 ml or ● 2 x 30 ml Buffer QC.**

    Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove all contaminants in the majority of plasmid DNA preparations. The second wash is particularly necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

    ☞ Remove a ▲ 200 µl or ● 120 µl sample from the combined wash fractions and save for an analytical gel (sample 3).

14. **Elute DNA with ▲ 5 ml or ● 15 ml Buffer QF.**

    Use of polycarbonate tubes (not supplied) to collect the eluate is not recommended as polycarbonate is not resistant the alcohol used in subsequent steps.

    Note: For constructs larger than 45–50 kb, prewarming the elution buffer to 65°C may help to increase yield.

    ☞ Remove a ▲ 50 µl or ● 30 µl sample of the eluate and save for an analytical gel (sample 4).

    ⊗ If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

15. **Precipitate DNA by adding ▲ 3.5 ml or ● 10.5 ml (0.7 volumes) of room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.**

    All solutions should be at room temperature in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. Alternatively, disposable conical bottom centrifuge tubes (not supplied) can be used for centrifugation at 5000 x *g* for 60 min at 4°C. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

16. **Wash DNA pellet with ▲ 2 ml or ● 5 ml room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    Alternatively, disposable conical-bottom centrifuge tubes (not supplied) can be used for centrifugation at 5000 x *g* for 60 min at 4°C. The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

17. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).**

    Redissolve DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under alkaline conditions; it does not easily dissolve in acidic buffers.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry and quantitative analysis on an agarose gel.

Agarose gel analysis

We recommend removing and saving aliquots during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (see page 41).

Troubleshooting Guide

Poor yields and quality can be caused by a number of different factors. For optimal plasmid preparation conditions, particular attention should be paid to the lysis conditions as described in the protocol. In addition, adhering to our recommendations with respect to plasmid copy number, capacity of QIAGEN-tip, culture volume, and culture media will ensure consistent and optimal results.

The following troubleshooting guide as well "General Considerations for Optimal Results" provided on our Web page www.qiagen.com/goto/plasmidinfo may be helpful in solving any problems that may arise. The scientists at QIAGEN Technical Service are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (see back cover for contact information).

		Comments and suggestions
Low or no DNA yield		
No DNA in lysate (sample 1)		
a)	Plasmid did not propagate	Please read "Growth of Bacterial Cultures" on our Web page www.qiagen.com/goto/plasmidinfo , and check that the conditions for optimal growth were met.
b)	Alkaline lysis was inefficient	If cells have grown to very high densities or a larger amount of cultured medium than recommended was used, the ratio of biomass to lysis reagent is shifted. This may result in poor lysis conditions, because the volumes of Buffers P1, P2, and P3 are not sufficient for setting the plasmid DNA free efficiently. Reduce culture volume or increase volumes of Buffers P1, P2, and P3. Also insufficient mixing of lysis reagents will result in reduced yield. Mix thoroughly after addition of Buffers P1, P2, and P3 to achieve homogeneous suspensions. Use LyseBlue to visualize efficiency of mixing.
c)	Insufficient lysis for low-copy plasmids	For low copy-plasmid preparations, doubling the volumes of lysis buffers P1, P2, and P3 may help to increase plasmid yield and quality (see page 29 and background on our Web page www.qiagen.com/goto/plasmidinfo).

		Comments and suggestions
d)	Lysate incorrectly prepared	Check Buffer P2 for SDS precipitation resulting from low storage temperatures and dissolve the SDS by warming. The bottle containing Buffer P2 should always be closed immediately after use. Lysis buffers prepared in the laboratory should be prepared according to the instructions on page 45. If necessary, prepare fresh Buffers P1, P2, and P3.
DNA in flow-though fraction (sample 2)		
a)	Column was overloaded	Check the culture volume and yield against the capacity of the QIAGEN-tip, as detailed at the beginning of each protocol. Reduce the culture volume accordingly, or select a larger QIAGEN-tip if a higher yield is desired. For very low-copy number plasmid and cosmid preps requiring very large culture volumes, please see page 29.
b)	SDS (or other ionic detergent) was in lysate	Chill Buffer P3 before use. If the lysate is cleared by centrifugation, load onto QIAGEN-tip promptly after centrifugation. If lysate is too viscous for effective mixing of Buffer P3, reduce culture volume or increase volumes of Buffers P1, P2, and P3. Use LyseBlue to visualize efficiency of mixing.
c)	Inappropriate salt or pH conditions in buffers	Ensure that any buffers prepared in the laboratory were prepared according to the instructions provided on page 45.
d)	Column flow was uneven	Store QIAGEN-tips at room temperature (15–25°C). If stored under cold, damp conditions for prolonged periods of time, the resin may clump. This problem can be overcome by shaking the column before use.
DNA in Buffer QC wash fraction (sample 3)		
a)	Column was overloaded	Check the culture volume and yield against the capacity of the QIAGEN-tip, as detailed at the beginning of each protocol. Reduce the culture volume accordingly, or select a larger QIAGEN-tip if a higher yield is desired. For very low-copy-number plasmid and cosmid preps requiring very large culture volumes, please see page 29.

		Comments and suggestions
b)	Buffer QC was incorrect	Check pH and salt concentration of Buffer QC. Recover DNA by precipitation, and purify on a new QIAGEN-tip as detailed in "Purification of plasmid DNA prepared by other methods" on our Web page www.qiagen.com/goto/plasmidinfo .

No DNA in eluate (sample 4)

a)	No DNA in the lysate	See section "No DNA in lysate" page 35.
b)	Elution Buffer QF or QN was incorrect	Check pH and salt concentration of Buffer QF or QN. Recover DNA by eluting with fresh buffer.
c)	DNA passed through in the flow-through or wash fraction	See previous two sections.

Little or no DNA after precipitation

a)	DNA failed to precipitate	Ensure that the precipitate is centrifuged at ≥15,000 x *g* for 30 min. Recover DNA by centrifuging for longer and at higher speeds. Try another isopropanol batch.
b)	DNA pellet was lost	Isopropanol pellets are glassy and may be difficult to see. Mark the outside of the tube before centrifugation. Isopropanol pellets may also be loosely attached to the side of the tube, so pour supernatant off gently.
c)	DNA was poorly redissolved	Check that DNA is completely redissolved. Be sure to wash any DNA off the walls, particularly if glass tubes and a fixed-angle rotor are used. Up to half of the total DNA may be smeared on the walls. Alternatively, a swinging bucket rotor can be used to ensure that the pellet is located at the bottom of the tube.

Plasmid DNA difficult to redissolve

a)	Pellet was overdried	Air-dry pellet instead of using a vacuum, especially if the DNA is of high molecular weight. Redissolve DNA by warming the solution slightly, and allowing more time for redissolving.
b)	Residual isopropanol in pellet	Ensure that pellets are washed with 70% ethanol to remove traces of isopropanol. Redissolve DNA by warming the solution slightly, and allowing more time for redissolving. Increase volume of buffer used for redissolving if necessary.

		Comments and suggestions
c)	Too much salt in pellet	Ensure that isopropanol is at room temperature for precipitation, and wash the pellet twice with room temperature 70% ethanol. Recover DNA by increasing the volume of buffer used for redissolving.
d)	Buffer pH was too low	Ensure that the pH of the buffer used for redissolving is ≥8.0, since DNA does not dissolve well in acidic solutions.
e)	Resuspension volume too low	Increase resuspension volume if the solution above the pellet is highly viscous.

Contaminated DNA/poor-quality DNA

a)	Genomic DNA in the eluate	Mixing of bacterial lysate was too vigorous. The lysate must be handled gently after addition of Buffers P2 and P3 to prevent shearing of chromosomal DNA. Reduce culture volume if lysate is too viscous for gentle mixing.
b)	RNA in the eluate	RNase A digestion was insufficient. Check culture volume against recommended volumes, and reduce if necessary. Check that the RNase A provided with the kit has been used. If Buffer P1 is more than 6 months old, add more RNase A. Recover DNA by precipitating the eluate, digesting with RNase A, and purifying on a new QIAGEN-tip as detailed in "Purification of plasmid DNA prepared by other methods" on our Web page www.qiagen.com/goto/plasmidinfo .
c)	Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use new glass- and plasticware, and wear gloves.
d)	Lysis time was too long	Ensure that lysis step (Buffer P2) does not exceed 5 min.
e)	Overloaded alkaline lysis	Check the culture volume and yield against the capacity of the QIAGEN-tip. Reduce the culture volume accordingly or alternatively increase the volumes of Buffers P1, P2, and P3.
f)	Plasmid DNA is nicked/sheared/ degraded	DNA was poorly buffered. Redissolve DNA in TE buffer, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.
g)	Endonuclease-containing host	Refer to background information on our Web page (www.qiagen.com/goto/plasmidinfo), and consider changing *E. coli* host strain.

		Comments and suggestions
h)	Shearing during redissolving	Redissolve DNA gently, without vortexing or vigorous pipetting. Avoid using small pipet tips.
i)	Particles in redissolved DNA	Centrifuge the DNA solution and transfer supernatant to a new tube. The particles have no affect on DNA quality. Alternatively, use HiSpeed kits containing QIAprecipitator, which filters the eluate.
Poor DNA performance		
a)	Too much salt in pellet	Ensure that isopropanol is at room temperature for precipitation, and wash the pellet twice with room temperature 70% ethanol. Precipitate the DNA again to remove the salt.
b)	Residual protein	Check culture volume against the recommended volumes and reduce if necessary. Ensure that the bacterial lysate is cleared properly by centrifugation at ≥20,000 x *g* for 45 min, or using a QIAfilter Cartridge.
Extra DNA bands on analytical gel		
a)	Dimer form of plasmid	Dimers or multimers of supercoiled plasmid DNA are formed during replication of plasmid DNA. Typically, when purified plasmid DNA is electrophoresed, both the supercoiled monomer and dimer form of the plasmid are detected upon ethidium bromide staining of the gel (see Figure 3, page 42). The ratio of these forms is often host dependent.
b)	Plasmid has formed denatured supercoils	This species runs faster than closed circular DNA on a gel and is resistant to restriction digestion (see Figure 3, page 42). Do not incubate cells for longer than 5 min in Buffer P2. Mix immediately after addition of Buffer P3.
c)	Possible deletion mutants	Some sequences are poorly maintained in plasmids. Check for deletions by restriction analysis. Cosmid clones, in particular, should always be prepared from freshly streaked, well-isolated colonies, since cosmids are not stable in *E. coli* for long periods of time.

	Comments and suggestions
Blocked QIAGEN-tip	
Lysate was turbid	Ensure that the lysate is clear before it is loaded onto the column. Ensure that Buffer P3 is chilled before use. Check *g*-force and centrifugation time. Alternatively, clear the lysate using a QIAfilter Cartridge. To clear a blocked QIAGEN-tip, positive pressure may be applied (e.g., by using a syringe fitted into a rubber stopper with a hole).

Appendix A: Agarose Gel Analysis of the Purification Procedure

DNA yields and quality can be readily analyzed by agarose gel electrophoresis. Poor yields and quality can be caused by a number of different factors. To determine at what stage of the procedure any problem occurred, save fractions from different steps of the purification procedure (see below and Table 4), and analyze by agarose gel electrophoresis.

Preparation of samples

Remove aliquots from the cleared lysate (sample 1), flow-through (sample 2), combined Buffer QC wash fractions (sample 3), and Buffer QF/QN eluate (sample 4), as indicated in each protocol and in Table 4. Precipitate the nucleic acids with 1 volume of isopropanol, rinse the pellets with 70% ethanol, drain well, and resuspend in 10 µl TE buffer, pH 8.0.

Table 4. Sample Volumes Required for Agarose Gel Analysis

							Very low-copy plasmids/cosmids	
							QIAGEN-tip	
Sample	**Protocol step**	**Mini**	**Midi**	**Maxi**	**Mega**	**Giga**	**100**	**500**
1	Cleared lysate	50 µl	240 µl	120 µl	120 µl	75 µl	600 µl	750 µl
2	Flow-through	50 µl	240 µl	120 µl	120 µl	75 µl	50 µl	24 µl
3	Combined wash fractions	220 µl	400 µl	240 µl	160 µl	120 µl	200 µl	120 µl
4	Eluate	45 µl	100 µl	60 µl	22 µl	20 µl	50 µl	30 µl
(% of prep represented by each sample volume)		5.50%	2.00%	0.40%	0.08%	0.02%	1.00%	0.20%

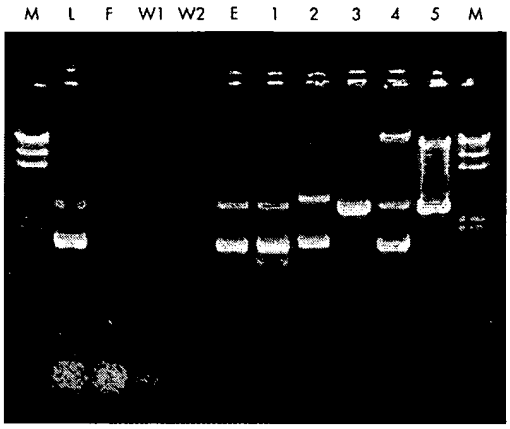


Figure 3 Agarose gel analysis of the plasmid purification procedure.

Agarose gel analysis

Run 2 µl of each sample on a 1% agarose gel* for analysis of the fractions at each stage of the plasmid purification procedure. Figure 3 shows an analytical gel of the different fractions, together with examples of problems that can arise at each step. If you find that you have a problem with a particular step of the protocol, turn to the hints in the relevant section of the troubleshooting guide on pages 35 – 40. If the problem remains unresolved, or if you have any further questions, please call QIAGEN Technical Service.

L: Cleared lysate containing supercoiled and open circular plasmid DNA and degraded RNA (sample 1).

F: Flow-through fraction containing only degraded RNA is depleted of plasmid DNA which is bound to the QIAGEN Resin (sample 2).

W1: First wash fraction, in which the remaining traces of RNA are removed without affecting the binding of the DNA (sample 3).

W2: Second wash fraction, which ensures that the resin is completely cleared of RNA and other contaminants, leaving only pure plasmid DNA on the column (sample 3).

E: The eluate containing pure plasmid DNA with no other contaminating nucleic acids (sample 4).

M: Lambda DNA digested with *Hin*dIII.*

Lanes 1–5 illustrate some atypical results that may be observed in some preparations, depending on plasmid type and host strain.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Lane 1: Supercoiled (lower band) and open circular form (upper band) of the high-copy plasmid pUC18 with an additional band of denatured supercoiled DNA migrating just below the supercoiled form. This form may result from prolonged alkaline lysis with Buffer P2 and is resistant to restriction digestion.

Lane 2: Multimeric forms of supercoiled plasmid DNA (pTZ19) which may be observed with some host strains, and should not be mistaken for genomic DNA. Multimeric plasmid DNA can easily be distinguished from genomic DNA by a simple restriction digestion: linearization of a plasmid sample displaying multimeric bands will yield a single defined band with the size of the linearized plasmid monomer (see lane 3).

Lane 3: Linearized form of plasmid pTZ19 after restriction digestion with *Eco*RI.

Lane 4: Sample contaminated with bacterial chromosomal DNA, which may be observed if the lysate is treated too vigorously (e.g., vortexing during incubation steps with Buffer P2 or P3). Genomic DNA contamination can easily be identified by digestion of the sample with *Eco*RI. A smear is observed, in contrast to the linear band seen after digestion of multimeric plasmid forms.

Lane 5: *Eco*RI digestion of a sample contaminated with bacterial genomic DNA which gives a smear above the plasmid DNA.

Appendix B: Composition of Buffers

Buffer	Composition	Storage
Buffer P1 (resuspension buffer)	50 mM Tris·Cl, pH 8.0; 10 mM EDTA; 100 µg/ml RNase A	2–8°C, after addition of RNase A
Buffer P2 (lysis buffer)	200 mM NaOH, 1% SDS (w/v)	15–25°C
Buffer P3 (neutralization buffer)	3.0 M potassium acetate, pH 5.5	15–25°C or 2–8°C
Buffer FWB2 (QIAfilter wash buffer)	1 M potassium acetate pH 5.0	15–25°C
Buffer QBT (equilibration buffer)	750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v); 0.15% Triton® X-100 (v/v)	15–25°C
Buffer QC (wash buffer)	1.0 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v)	15–25°C
Buffer QF (elution buffer)	1.25 M NaCl; 50 mM Tris·Cl, pH 8.5; 15% isopropanol (v/v)	15–25°C
Buffer QN (elution buffer)	1.6 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v)	15–25°C
TE	10 mM Tris·Cl, pH 8.0; 1 mM EDTA	15–25°C
STE	100 mM NaCl; 10 mM Tris·Cl, pH 8.0; 1 mM EDTA	15–25°C

Preparation of buffers

Buffer compositions are given per liter of solution. Do not autoclave MOPS- or isopropanol-containing buffers; sterilize by filtration instead.

Buffer calculations are based on Tris base adjusted to pH with HCl (Tris·Cl). If using Tris·HCl reagent, the quantities used should be recalculated.

P1: Dissolve 6.06 g Tris base, 3.72 g $Na_2EDTA{\cdot}2H_2O$ in 800 ml distilled water. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with distilled water. Add 100 mg RNase A per liter of P1.

P2: Dissolve 8.0 g NaOH pellets in 950 ml distilled water, 50 ml 20% SDS (w/v) solution. The final volume should be 1 liter.

P3: Dissolve 294.5 g potassium acetate in 500 ml distilled water. Adjust the pH to 5.5 with glacial acetic acid (~110 ml). Adjust the volume to 1 liter with distilled water.

FWB2: Dissolve 98.2 g potassium acetate in 500 ml distilled water. Adjust the pH to 5.0 with glacial acetic acid (~36 ml). Adjust the volume to 1 liter with distilled water.

QBT: Dissolve 43.83 g NaCl, 10.46 g MOPS (free acid) in 800 ml distilled water. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol and 15 ml 10% Triton X-100 solution (v/v). Adjust the volume to 1 liter with distilled water.

QC: Dissolve 58.44 g NaCl and 10.46 g MOPS (free acid) in 800 ml distilled water. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.

QF: Dissolve 73.05 g NaCl and 6.06 g Tris base in 800 ml distilled water and adjust the pH to 8.5 with HCl. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.

QN: Dissolve 93.50 g NaCl and 10.46 g MOPS (free acid) in 800 ml distilled water and adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.

STE: Dissolve 5.84 g NaCl, 1.21 g Tris base, and 0.37 g $Na_2EDTA{\cdot}2H_2O$ in 800 ml distilled water. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with distilled water.

Note: Always recheck pH of buffers after preparation.

Preparation of LB medium

Dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 800 ml distilled water. Adjust the pH to 7.0 with 1 N NaOH. Adjust the volume to 1 liter with distilled water. Sterilize by autoclaving.

Ordering Information

Product	Contents	Cat. no.
QIAGEN Plasmid Kits		
QIAGEN Plasmid Mini Kit (25)	25 QIAGEN-tip 20, Reagents, Buffers	12123
QIAGEN Plasmid Mini Kit (100)	100 QIAGEN-tip 20, Reagents, Buffers	12125
QIAGEN Plasmid Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers	12143
QIAGEN Plasmid Midi Kit (100)	100 QIAGEN-tip 100, Reagents, Buffers	12145
QIAGEN Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers	12162
QIAGEN Plasmid Maxi Kit (25)	25 QIAGEN-tip 500, Reagents, Buffers	12163
QIAGEN Plasmid Maxi Kit (100)	100 QIAGEN-tip 500, Reagents, Buffers	12165
QIAGEN Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers	12181
QIAGEN Plasmid Mega Kit (25)	25 QIAGEN-tip 2500, Reagents, Buffers	12183
QIAGEN Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, Buffers	12191
QIAfilter Plasmid Kits		
QIAfilter Plasmid Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12243
QIAfilter Plasmid Midi Kit (100)	100 QIAGEN-tip 100, Reagents, Buffers, 100 QIAfilter Midi Cartridges	12245
QIAfilter Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers, 10 QIAfilter Maxi Cartridges	12262
QIAfilter Plasmid Maxi Kit (25)	25 QIAGEN-tip 500, Reagents, Buffers, 25 QIAfilter Maxi Cartridges	12263

Ordering Information

Product	Contents	Cat. no.
QIAfilter Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12281
QIAfilter Plasmid Giga Kit (5)*	5 QIAGEN-tip 10000, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12291
HiSpeed Plasmid Kits		
HiSpeed Plasmid Midi Kit (25)	25 HiSpeed Midi Tips, 25 QIAfilter Midi Cartridges, 25 QIAprecipitator Midi Modules plus Syringes, Reagents, Buffers	12643
HiSpeed Plasmid Maxi Kit (10)	10 HiSpeed Maxi Tips, 10 QIAfilter Maxi Cartriges, 10 QIAprecipitator Maxi Modules plus Syringes, Reagents, Buffers	12662
Hispeed Plasmid Maxi Kit (25)	25 HiSpeed Maxi Tips, 25 QIAfilter Maxi Cartriges, 25 QIAprecipitator Maxi Modules plus Syringes, Reagents, Buffers	12663
EndoFree Plasmid Kits		
EndoFree Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, 10 QIAfilter Maxi Cartridges, Endotoxin-free Buffers	12362
EndoFree Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, 5 QIAfilter Mega-Giga Cartridges, Endotoxin-free Buffers	12381
EndoFree Plasmid Giga Kit (5)*	5 QIAGEN-tip 10000, Reagents, 5 QIAfilter Mega-Giga Cartridges, Endotoxin-free Buffers	12391

* For purification of low-copy plasmids and cosmids, QIAfilter Plasmid Mega Kits are a better choice than QIAfilter Plasmid Giga Kits, due to the large culture volumes required and the limited capacity of the QIAfilter Mega-Giga Cartridge.

Ordering Information

Product	Contents	Cat. no.
QIAprep Spin Kits		
QIAprep Spin Miniprep Kit (50)	50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27104
QIAprep Spin Miniprep Kit (250)	250 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27106
CompactPrep Plasmid Kits*		
CompactPrep Plasmid Midi Kit (25)*	25 CompactPrep Midi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12743
CompactPrep Plasmid Midi Kit (100)*	100 CompactPrep Midi Columns, Extender Tubes, Reagents, Buffers, 100 QIAfilter Midi Cartridges	12745
CompactPrep Plasmid Maxi Kit (25)*	25 CompactPrep Maxi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Maxi Cartridges	12763
CompactPrep Plasmid Maxi Kit (100)*	100 CompactPrep Maxi Columns, Extender tubes, Reagents, Buffers, 100 QIAfilter Maxi Cartridges	12765
CompactPrep Plasmid Midi Core Kit (100)*	100 CompactPrep Midi Columns, Extender tubes, Reagents, Buffers	12845
CompactPrep Plasmid Maxi Core Kit (100)*	100 CompactPrep Maxi Columns, Extender tubes, Reagents, Buffers	12865
QIAGEN Large-Construct Kit		
QIAGEN Large-Construct Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers, ATP-Dependent Exonuclease†	12462

* CompactPrep Kits require use of a vacuum device for operation (e.g., QIAvac 24 Plus, cat. no. 19413).

† ATP solution for exonuclease digestion is not provided.

Ordering Information

Product	Contents	Cat. no.
Transfection products		
PolyFect Transfection Reagent (1 ml)	For 25–65 transfections in 60 mm dishes or 50–100 transfections in 6-well plates	301105
Effectene Transfection Reagent (1 ml)	For 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301425
SuperFect Transfection Reagent (1.2 ml)	For 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301305
Accessories		
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings	19413
QIArack	1 rack for 12 x QIAGEN-tip 20, 8 x QIAGEN-tip 100, 6 x QIAGEN-tip 500 or HiSpeed Midi Tips, 4 x QIAGEN-tip 2500 or HiSpeed Maxi Tips, and 10 QIAfilter Midi or Maxi Cartridges	19015
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml, solution)	19101
Plasmid Buffer Set	Buffers P1, P2, P3, QBT, QC, QF, RNase A; for 100 plasmid mini-, 25 midi-, or 10 maxipreps	19046
EndoFree Plasmid Buffer Set	Buffers P1, P2, P3, QBT, QC, QN, ER, TE, Endotoxin-free water, RNase A; for 10 plasmid mega- or 5 gigapreps (endotoxin-free)	19048
Buffer P1	500 ml Resuspension Buffer (RNase A not included)	19051
Buffer P2	500 ml Lysis Buffer	19052
Buffer P3	500 ml Neutralization Buffer	19053

Notes

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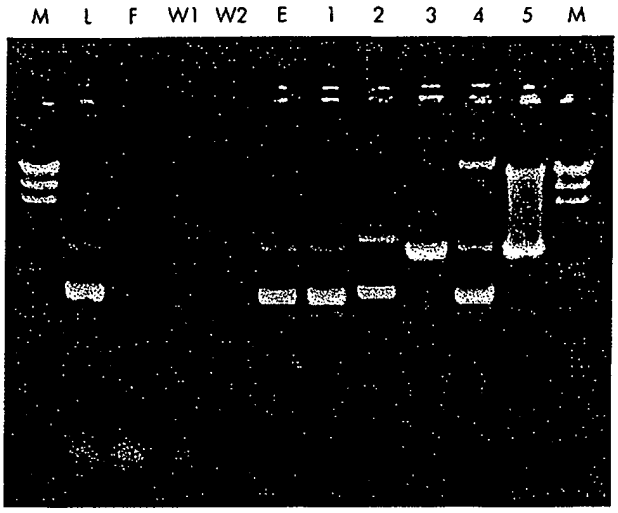

Figure 3 Agarose gel analysis of the plasmid purification procedure.

Agarose gel analysis

Run 2 µl of each sample on a 1% agarose gel* for analysis of the fractions at each stage of the plasmid purification procedure. Figure 3 shows an analytical gel of the different fractions, together with examples of problems that can arise at each step. If you find that you have a problem with a particular step of the protocol, turn to the hints in the relevant section of the troubleshooting guide on pages 35 – 40. If the problem remains unresolved, or if you have any further questions, please call QIAGEN Technical Service.

L: Cleared lysate containing supercoiled and open circular plasmid DNA and degraded RNA (sample 1).

F: Flow-through fraction containing only degraded RNA is depleted of plasmid DNA which is bound to the QIAGEN Resin (sample 2).

W1: First wash fraction, in which the remaining traces of RNA are removed without affecting the binding of the DNA (sample 3).

W2: Second wash fraction, which ensures that the resin is completely cleared of RNA and other contaminants, leaving only pure plasmid DNA on the column (sample 3).

E: The eluate containing pure plasmid DNA with no other contaminating nucleic acids (sample 4).

M: Lambda DNA digested with *Hin*dIII.*

Lanes 1–5 illustrate some atypical results that may be observed in some preparations, depending on plasmid type and host strain.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.